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Safety of immunotherapy with *Alternaria alternata* glutaraldehyde-polymerized allergen extract in adults and children

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Abstract
Background: Allergies to fungi, such as *Alternaria alternata*, are significant contributors to respiratory conditions like asthma and rhinitis. Immunotherapy with native *A. alternata* extracts often results in high rates of adverse reactions. This study evaluates the safety of immunotherapy using glutaraldehyde-polymerized *A. alternata* extracts in both pediatric and adult populations.
Methods: This observational real-world study involved 738 patients (435 children and 303 adults) monosensitized to *A. alternata* or polysensitized together to other allergens. Patients received personalized immunotherapy containing polymerized *A. alternata* extract, either alone or in combination with other polymerized allergens. The concentration of each polymerized allergen in the therapeutic vaccine was 10,000 TU/mL. Side reactions were classified and recorded based on immediate and delayed onset, and their severity was graded according to the EAACI guidelines.
Results: All side reactions were expected and related to the intrinsic properties of allergens. The number of injections administered was 7392, with 4137 to children and 3255 to adults. Thirteen relevant local reactions (0.24% in children, 0.09% in adults) and seven systemic reactions (0.09% overall) were observed. Systemic reactions included mild to moderate symptoms, such as mild bronchospasm and rhinitis. Severe reactions were not reported.
Conclusion: Immunotherapy with glutaraldehyde-polymerized *A. alternata*, alone or in combination with other polymerized allergens, is safe and well-tolerated in both children and adults. The polymerized extracts allow for higher concentrations and faster administration schedules, providing an effective treatment option for patients with fungal allergies, including those polysensitized to multiple allergens.
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Introduction

Fungi are relevant in allergy due to not only the sensitization rates but also the symptomatology associated with the allergic disease.[1] The prevalence of fungal sensitization is not well-defined, although it is estimated to affect between 3 and 10% of the world's population, 12–42% of atopic patients, and more than 66% of patients with severe asthma.[2] Fungal sensitization varies according to age and country.[2] In Alergologica 2015 study (Spain), 10.1 % of subjects with extrinsic asthma had positive test results for molds.[3] In the Escape (Europe) study on pediatric asthma, sensitization was estimated to be 17.7%.[4] In Poland, 22.17% of adult patients were allergic to fungi, with *Alternaria* spp. (47.1%) and *Cladosporium* spp. (30.8%) being the most frequent allergen sources.[5] In the United States, the prevalence of *A. alternata* sensitization in the general population was 13%,[6] and in the Beijing area (China), fungi were one of the most common aeroallergens of respiratory allergy in children.[7] A study conducted in Germany from 2008 to 2011 assessed the sensitization prevalence and profile of the population allergic to fungi, revealing that the studied population was sensitized to *A. alternata* (3.0%), *Aspergillus fumigatus* (2.3%), or *Cladosporium herbarum* (1.3%). In this study, the sensitization data (using skin prick test) from 1998 to 2007 and 2008 and 2017, were also analyzed, retrospectively, and an increased prevalence of these main fungal allergenic species in the second decade was noted.[8]

Alternaria alternata allergy is an important risk factor for severe asthma, especially in children and young people. It is reported that 40% of asthmatic children are sensitive to fungi, but the prevalence increases above 60% in children with severe asthma, and in most of cases this pathology persists into adulthood.[9]

Immunotherapy with native allergen extracts of *A. alternata* has been demonstrated to provide clinical improvement in adults and children.[10] Prospective, double-blind trials conducted so far have shown a symptomatological improvement in treated patients, as well as a decrease in IgE antibody levels, and an increase in serum IgG levels.[11] Nevertheless, one of the main drawbacks of fungal immunotherapy with native extracts is the safety profile, as immunotherapy with these extracts has been associated with a higher incidence of local and systemic adverse reactions than other allergen extracts.[12] In addition, most subjects with allergies to molds are usually sensitized to other aeroallergens.[2] Therefore, to experience clinical improvement, immunotherapy against the allergens to which they are allergic is needed. This can be achieved by using several individual immunotherapies or one containing a mixture of all clinically relevant components in the appropriate concentration.

Polymerization of allergens with glutaraldehyde allows for a reduction in the risk of adverse reactions associated with allergen immunotherapy,[13,14] providing allergoids with decreased IgE-binding capacity. Additionally, polymerization has been demonstrated to reduce the enzymatic proteolytic activity, which assures the integrity of therapeutic vaccine components[15] and abolishes the bias of a Th2 immune response.[16–18]

Currently, the percentage of polysensitized patients is growing, and in the case of patients allergic to *A. alternata*, the reality is not different, in which most patients with mould allergy are often sensitized to other allergenic sources[2] and usually require treatment with therapeutic allergen vaccines containing allergen mixtures.[19,20] This approach has several difficulties, including incompatibilities that may exist between extracts, particularly by the presence of proteolytic enzymes in at least one allergenic source.

Previous experience with glutaraldehyde-polymerized allergen extracts, administered as a single component or as mixtures with other polymerized allergen extracts, without dilution in the mixture (Clustoid® Max) has demonstrated to be safe. In this scenario of an allergen source with high clinical relevance, whose sensitization is increasing and, in most cases, coexists with others, and whose immunotherapy is classically associated with an increased risk of adverse effects, this study had the objective to evaluate, in real-world conditions, the safety of immunotherapy with a glutaraldehyde-polymerized *A. alternata* allergen extract with or without a mixture with other polymerized allergen extracts.

Materials and Methods

Design and ethics

The study was classified as an Observational Postauthorization by the Spanish Health Authorities (Agencia Española de Medicamentos y Productos Sanitarios) and was evaluated and authorized by the ethics committee of the "Comunidad Autónoma de Madrid." Forty-six specialist physicians participated in this study.

Patient population

Data were collected from the medical records of 738 subjects (457 males and 281 females) sensitized to *A. alternata* or polysensitized to *A. alternata* and other allergens (house dust mites, grass pollen, Salsola pollen and/or Olea pollen). Of these, 435 were pediatric patients (59%) aged 0–17 years with a mean age of 9.5 years (95% confidence interval 9.2–9.8), and 303 patients were adults with a mean age of 33.3 years old (95% confidence interval 27.5–39.2). Table 1 shows the epidemiological data, including other sensitizations.

Immunotherapy preparation

Each patient received an individualized therapeutic vaccine of *A. alternata* extract containing all relevant allergens, polymerized with glutaraldehyde and adsorbed onto aluminum hydroxide, alone or in a mixture without dilution with other polymerized allergenic extracts (*Dermatophagoides*, grass pollen, Salsola pollen and/or Olea pollen) (Clustoid® Max Alternaria, Inmunotek, SL, Alcalá de Henares, Spain).

The concentration of each polymerized allergen in the therapeutic vaccine was 10,000 TU/mL (TU: Therapeutic units). The composition of the treatments is described in Table 2.

Table 1 Epidemiological data of subjects.

		Paediatric					
		Children		Adolescents			
All		(5 -12 years)		(13-17 years)		Adults	
n	738	253	34%	182	25%	303	41%
Male	457	179	24%	107	14%	171	23%
Female	281	74	10%	75	10%	132	18%

Table 2 Composition of the treatments.

		Paediatric		
	All	Children (5-12 years)	Adolescents (13-17)	Adults
A. alternata	167	44	42	81
A. alternata + *Dermatophagoides**	123	39	33	51
A. alternata + *Dermatophagoides** + *O. europaea*	31	11	9	11
A. alternata + *Dermatophagoide*s* + *grasses*	28	9	4	15
A. alternata + *grasses*	61	12	13	36
A. alternata + *grasses* + *O. europaea*	155	71	35	49
A. alternata + *O. europaea*	147	61	36	50
A. alternata + *O. europaea* + *S. kali*	1	-	-	1
Alternara + *S. kali*	25	6	10	9
Total	738	253	182	303

**Dermatophagoides: D. pteronyssinus + D. farinae*

Different treatment schedules were used, the most common being the conventional, rush, and ultra-rush schedules.

In conventional schedules (n = 81), the patient received an injection of 0.2 mL and after a week 0.5 mL. In rush schedule (n = 638), the first dose of 0.2 mL was injected in one arm and 0.3 mL 30 min later in the other arm. In the ultra-rush schedule (n = 22), the first injection was a maintenance dose of 0.5 mL. Other schedules were used in 22 patients.

Safety assessment

Adverse reactions were classified as a) immediate, when the onset occurred during the first 30 min post-injection, and b) delayed, when the onset of the symptoms occurred afterwards.[21]

Local subcutaneous (SC) reactions were quantified by measuring the diameter of the induration. Immediate SC reactions with a diameter less than 5 cm and delayed reactions less than 10 cm were considered clinically irrelevant.[22] Systemic reactions were graded according to the EAACI Position Paper[21]: a) Grade 0: No symptoms or non-specific symptoms; b) Grade 1: Mild systemic reactions. Symptoms included localized urticaria, rhinitis, or mild asthma (Peak flow [PF] <20% decreased from baseline); c) Grade 2: Moderate systemic reactions. Symptoms include slow onset (>15 min) of generalized urticaria and/or moderate asthma (PF > 40% decrease from baseline); d) Grade 3: Severe (non-life-threatening) systemic reactions. Symptoms include rapid onset (<15 min) of generalized urticaria, angioedema, or severe asthma (PF > 40% decrease from baseline); e) Grade 4: Anaphylactic shock. Symptoms include the immediate evoked reaction of itching, flushing, erythema, generalized urticaria, stridor, immediate asthma, and hypotension.

Sample size determination

The sample size calculation was based on the outcomes of the study of Martínez-Cañavate et al.,[23] in which 12 adverse reactions were observed in 94 subjects treated with an unmodified allergenic extract of *A. alternata*, who received a total of 952 SC injections, which implies 1.2 systemic reactions for every 100 injections. Considering that the rate of expected systemic reactions with polymerized extracts is four times lower, the number of injections that should be evaluated is a minimum of 2874, with a relative risk of 0.25 and f (error protection) of 7.84. Expecting that each patient received six injections, the minimum number of patients to evaluate was 500.

Results

Number of injections

A total of 7392 injections were administered: 4137 (56%) to children and 3255 (44%) to adults. Each patient received a mean of 9.5 injections.

Adverse reactions

There were no records related to unexpected adverse reactions, such as of toxic or infectious origins. All reactions were expected, and all due to the intrinsic properties of the allergen extracts.

A total of 13 relevant local reactions (9 immediate and 4 delayed) were recorded (1 per 56 patients, and 1 per 568 injections). Of these, 10 of the reactions (0.24% of injections, 6 immediate and 4 delayed) occurred in children, and the other 3 (0.09% of injections, all immediate reactions) occurred in adults.

There were seven systemic reactions in the 738 patients treated (0.95%, 4 in children and 3 in adults), and 7392 administrations (0.095%). Two of the reactions were immediate (Grade 2, bronchospasm) and five were delayed (2 in children and 3 in adults, all of them were Grade 1). The relationship between the two late reactions and the administration of immunotherapy is not clear, as one of the reactions consisted of general discomfort and the other of low-grade fever and local edema.

Regarding the relationship between systemic reactions and the composition of the therapeutic vaccine (*A. alternata* alone or in mixture with other polymerized allergens), four of the systemic reactions observed in two patients occurred in the treatment with *A. alternata* alone, and another systemic reaction occurred with the mixture *Alternaria* + grasses + *Olea europaea*, one with *Alternaria* + *Dermatophagoides* + *O. europaea*, and another with *Alternaria* + *Dermatophagoides* (Tables 3–5).

Table 3 Adverse local and systemic relevant reactions in children and adults.

Adverse reaction	Type reaction	Paediatric population	Adults	Total
Relevant local reactions	Immediate	6	3	9
	% patients	1.37	1	1.2
	% injection	0.14	0.09	0.12
	Delayed	4	-	4
	% patients	0.9	-	0.5
	% injection	0.01	-	0.05
Systemic reactions	Immediate	2	-	2
	% patients	0.46	-	0.27
	% injection	0.05	-	0.03
	Delayed	2	3	5
	% patients	0.46	1	0.68
	% injection	0.04	0.01	0.07

Discussion

The study presented here analyses the safety of Clustoid Max *Alternaria*, both in a single immunotherapy using glutaraldehyde-polymerized *A. alternata* and in a mixture with other different polymerized allergens (77% of the treatments studied), with each allergen at a concentration of 10,000 UT/mL.

Retrospective data related to the administration of vaccines containing polymerized allergen extracts, from the medical records of a cohort of 738 patients, was analyzed to evaluate the safety of these preparations. A large number of centers (46) were included to be more representative of real-world conditions and to avoid the bias of a single or few centers administering the treatment. All adverse reactions were allergic, expected during the use of specific immunotherapy.

The treatment with these glutaraldehyde polymerizates has shown an excellent safety profile in both children and adults in routine clinical practice, even with accelerated build-up administration regimens. The percentage of

Table 4 Description of systemic reactions.

Patient ID	Age	Composition	Type reaction	Dose (mL)	Symptoms	Grade
238	23	*Alternaria alternata*	Delayed	0.2	Rhinitis	1
238	23	*Alternaria alternata*	Delayed	0.2	Rhinitis	1
295	13	*Alternaria alternata*	Immediate	0.5	Bronchospasm	2
295	13	*Alternaria alternata*	Immediate	0.5	Bronchospasm	2
326	12	*Alternaria alternata*+ *grasses*+ *O. europaea*	Delayed	0.5	Malaise	Inespecific
423	8	*Alternaria alternata* + *Dermatophagoides**+ *O. europaea*	Delayed	0.5	Fever and local edema	1
729	46	*Alternaria alternata* + *Dermatophagoides**	Delayed	0.2	Pharyngeal pruritus	1

**Dermatophagoides: D. pteronyssinus + D. farinae*

Table 5 Description of local reactions.

Patient ID	Age	Composition	Type reaction	Dose (mL)	Diameter (cm)
103	13	*Alternaria*	Delayed	0.5	>10
155	10	*Alternaria* + *Dermatophagoides**	Delayed	0.5	>10 (cellulitis)
343	13	*Alternaria*	Delayed	0.5	10
412	12	*Alternaria*	Immediate	0.5	5
412	12	*Alternaria*	Immediate	0.5	5
427	36	*Alternaria*+ *grasses*+ *Dermatophagoides**	Immediate	0.5	6
432	29	*Alternaria* + *grasses*+ *Dermatophagoides**	Immediate	0.5	6
432	29	Alternaria + grasses+ Dermatophagoides*	Immediate	0.5	6
602	12	*Alternaria* + grasses+ *Olea*	Immediate	0.5	5
654	12	*Alternaria*+ *Dermatophagoides** +*Olea*	Immediate	0.2	5
654	12	*Alternaria*+ *Dermatophagoides** +*Olea*	Immediate	0.5	6
666	9	*Alternaria*	Immediate	0.5	5
711	9	*Alternaria* + *Dermatophagoides***	Delayed	0.3	15

**Dermatophagoides: D. pteronyssinus + D. farinae*

local reactions per administration was found to be 0.24% in children, whereas in adults it occurred in less than 0.1% of injections, without serious reactions.

Looking at systemic reactions, the rate of reactions was also minimal, with 0.09% of administrations having such reactions, all being of Grade 1 or 2, and easily treatable without the need to discontinue immunotherapy treatment.

These data are superior to those reported in previous studies of specific immunotherapy with this mold. In the study by Tabar et al.[24] published in the year 2000, conducted in Spain involving immunotherapy with nonmodified *A. alternata* allergens, 1.95% of administrations, affecting 39.5% of the patients, resulted in adverse reactions. Most of these reactions were systemic, involving the respiratory tract. Furthermore, the study indicated that children and asthmatic patients had a higher risk of adverse reactions. Notably, 7 out of 38 children discontinued treatment due to Grade III systemic reactions. Five years later, the Spanish Pediatric Allergology Society published a study where the incidence of adverse reactions in children receiving immunotherapy with *A. alternata* was lower (0.95% of the administered doses).[23] These figures are still higher than those presented in our study, in which the incidence of adverse reactions due to the administration of immunotherapy is 0.27%.

While immunotherapy with native preparations of *A. alternata* has traditionally presented safety challenges, this polymerized extract has shown to have a favorable safety profile with a notably low rate of adverse reactions, even when administered in combination with other extracts without dilution. The study demonstrates that there are no significant differences in safety between pediatric and adult populations. Furthermore, this immunotherapy facilitates rapid build-up schedules without compromising safety.

Conflicts of interest

All authors are employees of Inmunotek, S.L.

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Institutional Review Board Statement

The study was conducted per the Declaration of Helsinki and approved by the Ethics Committee of "Comunidad Autónoma de Madrid."

Informed Consent Statement

Patient consent was waived due to the retrospective nature of the study.

Data Availability Statement

The data presented in this study are available after request and permission.

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References

1. Crameri R, Garbani M, Rhyner C, Huitema C. Fungi: The neglected allergenic sources. Allergy. 2014;69(2):176–85. https://doi.org/10.1111/all.12325
2. Rick EM, Woolnough K, Pashley CH, Wardlaw AJ. Allergic fungal airway disease. J Investig Allergol Clin Immunol. 2016;26(6):344–54. https://doi.org/10.18176/jiaci.0122
3. Ojeda P, Sastre J, Olaguibel JM, Chivato T, Investigators participating in the National Survey of the Spanish Society of Allergology and Clinical Immunology Alergológica 2015. Alergológica 2015: A national survey on allergic diseases in the adult Spanish population. J Investig Allergol Clin Immunol. 2018;28(3):151–64. https://doi.org/10.18176/jiaci.0264
4. Martínez-Cañavate A, Valenzuela-Soria A, Rojo-Hernández A. Immunotherapy with *Alternaria alternata*: Present and future. Allergol Immunopathol (Madr). 2007;35(6):259–63. https://doi.org/10.1157/13112993
5. Bogacka E, Nittner-Marszalska M, Fal AM, Kuzniar J, Nikiel E, Malolepszy J. [Allergy to mould allergens as a risk factor for bronchial asthma in patients suffering from allergic rhinitis]. Pol Merkur Lekarski. 2003;14(83):388–92.
6. Arbes SJ, Jr., Gergen PJ, Elliott L, Zeldin DC. Prevalences of positive skin test responses to 10 common allergens in the US population: Results from the third National Health and Nutrition Examination Survey. J Allergy Clin Immunol. 2005;116(2):377–83. https://doi.org/10.1016/j.jaci.2005.05.017
7. Pang C, Bian SN, Liu CH, Guo LL, Cui Y, Lin F, et al. [The characteristics and change of aeroallergens in children from 2015 to 2020 in a hospital of pediatric in Beijing]. Zhonghua Yu Fang Yi Xue Za Zhi. 2021;55(7):840–6.
8. Forkel S, Beutner C, Schröder SS, Bader O, Gupta S, Fuchs T, et al. Sensitization against fungi in patients with airway allergies over 20 years in Germany. Int Arch Allergy Immunol. 2021;182(6):515–23. https://doi.org/10.1159/000512230
9. Vicencio AG, Santiago MT, Tsirilakis K, Stone A, Worgall S, Foley EA, et al. Fungal sensitization in childhood persistent asthma is associated with disease severity. Pediatr Pulmonol 2014; 49(1):8-14. https://doi.org/10.1002/ppul.22779
10. Bozek A, Pyrkosz K. Immunotherapy of mold allergy: A review. Hum Vaccin Immunother. 2017;13(10):2397–401. https://doi.org/10.1080/21645515.2017.1314404
11. Kuna P, Kaczmarek J, Kupczyk M. Efficacy and safety of immunotherapy for allergies to *Alternaria alternata* in children. J Allergy Clin Immunol. 2011;127(2):502–508 e1-6. https://doi.org/10.1016/j.jaci.2010.11.036
12. Helbling A, Reimers A. Immunotherapy in fungal allergy. Curr Allergy Asthma Rep. 2003;3(5):447–53. https://doi.org/10.1007/s11882-003-0082-x
13. Patterson R, Suszko IM, Zeiss CR, Pruzansky JJ. Development and evaluation of polymerized allergens for immunotherapy. Int Arch Allergy Appl Immunol. 1981;66 Suppl 1:293–6. https://doi.org/10.1159/000232924

14. Grammer LC, Shaughnessy MA, Patterson R. Modified forms of allergen immunotherapy. J Allergy Clin Immunol. 1985;76:397-401. https://doi.org/10.1016/0091-6749(85)90661-X
15. Tudela JI, Cases B, Fernández EA, Pastor C, Casanovas M, Subiza JL, et al. Enzymatic activity in native and polymerised mite allergen extracts. Allergy. 2012;67 (s96):242.
16. Gough L, Campbell E, Bayley D, Van Heeke G, Shakib F. Proteolytic activity of the house dust mite allergen Der p 1 enhances allergenicity in a mouse inhalation model. Clin Exp Allergy. 2003;33(8):1159-63. https://doi.org/10.1046/j.1365-2222.2003.01716.x
17. King C, Brennan S, Thompson PJ, Stewart GA. Dust mite proteolytic allergens induce cytokine release from cultured airway epithelium. J Immunol. 1998;161(7):3645-51. https://doi.org/10.4049/jimmunol.161.7.3645
18. Schulz O, Sewell HF, Shakib F. Proteolytic cleavage of CD25, the alpha subunit of the human T cell interleukin 2 receptor, by Der p 1, a major mite allergen with cysteine protease activity. J Exp Med. 1998;187(2):271-5. https://doi.org/10.1084/jem.187.2.271
19. Cox L, Calderon MA. Subcutaneous specific immunotherapy for seasonal allergic rhinitis: A review of treatment practices in the US and Europe. Curr Med Res Opin. 2010;26(12):2723-33. https://doi.org/10.1185/03007995.2010.528647
20. Migueres M, Dávila I, Frati F, Azpeitia A, Jeanpetit Y, Lhéritier-Barrand M, et al. Types of sensitization to aeroallergens: Definitions, prevalences and impact on the diagnosis and treatment of allergic respiratory disease. Clin Transl Allergy 2014; 4:16. https://doi.org/10.1186/2045-7022-4-16
21. Álvarez-Cuesta E, Bousquet J, Canonica GW, Durham SR, Malling HJ, Valovirta E. Standards for practical allergen-specific immunotherapy. Allergy. 2006;61 Suppl 82:1-20. https://doi.org/10.1111/j.1398-9995.2006.01219_1.x
22. Álvarez-Cuesta E, Boquete M, Cadahía A, Carrillo T, Fernández-Távora L, Hernández J, et al. Sociedad Española de Alergología e Inmunología Clínica. Normativa sobre Inmunoterapia en Enfermedades Alérgicas. Madrid: SANED; 1990.
23. Martínez-Cañavate A, Eseverri JL, Ródenas R, Tabar AI, Garde J, Torres J, et al. Evaluation of paediatric tolerance to an extract of *Alternaria alternata* under two treatment regimes. A multicentre study. Allergol Immunopathol (Madr). 2005;33(3):138-41. https://doi.org/10.1157/13075696
24. Tabar AI, Lizaso MT, García BE, Echechipía S, Olaguibel JM, Rodríguez A. Tolerance of immunotherapy with a standardized extract of *Alternaria tenuis* in patients with rhinitis and bronchial asthma. J Investig Allergol Clin Immunol. 2000;10(6):327-33.